Online supporting material

Supplemental methods

Pharmacokinetic data analysis (full description)

We analyzed the data using the software package NONMEM (non-linear mixed effects modeling) version VI 2.0, running with NM-TRAN. We applied subroutines ADVAN 1 to 6 and the first-order conditional estimation method (FOCE) with INTERACTION. We determined the appropriate structural model by fitting the pharmacokinetic data to one-, two-, and three-compartment models with zero-order or first-order absorption, and we estimated clearance (CL), central volume of distribution (V₁), intercompartmental clearance (Q), volume of the peripheral compartment (V₂), duration of the zero-order absorption process (D₁) or rate constant for the first-order (kₐ), and relative bioavailabilities (F). We compared various models with random interindivudual variability on several parameters. The statistical model used for describing the residual intraindividual variability was a combined exponential and additive model with both residual interindivudual errors considered normally distributed with mean 0. The change in the objective function (OF) resulting from the inclusion of additional parameters approximates a χ² distribution and the observed values were therefore regarded as statistically significant (P < 0.05) if they exceeded the critical values of χ² for the corresponding degrees of freedom, namely 3.84 for one, 5.99 for two, 7.81 for three, and 9.49 for four additional parameters.

In a first step, we analyzed the serum 25(OH)D data to obtain an appropriate model that predicted for each individual the time course of serum 25(OH)D during the study. Estimates of vitamin D intake through diet and supplementation and vitamin D synthesis from UV exposure provided the basis for the evaluation and comparison of several compartmental models for describing serum 25(OH)D pharmacokinetics. Second, we used the urinary ⁴¹Ca data from the labeling period to adapt the ⁴¹Ca model we previously described in a similar
population of older Swiss women to generate a revised model that predicted plasma $^{41}$Ca concentrations for the present cohort during the intervention period in the absence of vitamin D supplementation. We calculated $^{41}$Ca plasma concentrations by multiplying the urinary $^{41}$Ca/$^{40}$Ca ratios by 1.2 mmol/L, based on the mean concentration of plasma Ca (3). Finally, we incorporated the serum 25(OH)D model into the $^{41}$Ca model, fixed the pharmacokinetic parameters for $^{41}$Ca, and then allowed the individual predicted increases in serum 25(OH)D concentrations from supplement administration to influence one or several $^{41}$Ca transfer rates during the intervention period. We analysed the most likely candidate in detail and used the resulting model to predict the effect of vitamin D supplementation on serum 25(OH)D concentrations and $^{41}$Ca pharmacokinetics. We modelled the pharmacodynamic effect of the serum 25(OH)D concentration on Ca pharmacokinetics by using a maximal effect ($E_{\text{max}}$) model, in which $C_{50}$ corresponds to the increase in serum 25(OH)D concentrations leading to the half-maximal effect. We chose the $E_{\text{max}}$ model over a linear model because the pharmacodynamic effect was found to plateau with increasing serum 25(OH)D concentration.

We then plotted and compared predictions for $^{41}$Ca concentrations, with and without incorporation of vitamin D supplementation into the model. Finally, we refitted the model with the complete new data set including all $^{41}$Ca measurements from the labeling and the intervention period, and serum 25(OH)D concentrations as predicted from our vitamin D model. We used Pearson product-moment correlation coefficient (Pearson's r) as a measure of the strength of linear dependence between two variables.

**Supplemental results**

Compartmental analysis of vitamin D pharmacokinetics (full description)

We started the analysis with a one compartment linear model with interindividual variability on clearance (supplemental Table 1). The inclusion of interindividual variability on the volume of distribution resulted in a significantly better model fit and decreased the objective
function (OF) by 10. The inclusion of a first order absorption compartment for estimated vitamin D synthesis from sun exposure did not improve this model and absorption was estimated to be immediate (absorption rate $k_a = 4 \times 10^7 \text{d}^{-1}$). Similarly, inclusion of the estimated bioavailability for dietary vitamin D intake along with sun exposure did not significantly improve the model (supplemental Table 1). However, inclusion of a second compartment significantly decreased the OF by 20. We found no improvement of fit when comparing individual and population predictions for both models with the observed data. Because of the increase in model complexity with two additional fixed and two additional random effects parameters without any visible improvement in data fit, we discarded the two-compartment model and proceeded to the calcium data using the one-compartment serum 25(OH)D model. **Supplemental Figure 1** shows the population and individual predictions of serum 25(OH)D concentrations for this final model for three representative subjects with good, average and poor fit, selected at the 10th, 50th, and 90th percentile of the mean squared difference between observed and predicted serum 25(OH)D concentrations. **Table 2** in the main text shows the predicted serum 25(OH)D concentrations using the model next to the measured values for time points before and after each intervention period.

*Model for calcium distribution and excretion (full description)*

We combined data from our previous $^{41}$Ca study (3) with the data of the present study to refit our previous model (3) and predict calcium distribution, excretion, and plasma $^{41}$Ca concentrations after the labelling period in the absence of vitamin D supplementation. The new three compartment model, schematically represented in **Figure 1** in the main text, described both the old and new $^{41}$Ca datasets well (compare **Supplemental Table 2**). Comparing the models, the difference in parameter volume $V_1$, (65.5 L vs. 18.9 L) is explained by the lower bioavailability of the oral dose in the previous study (3) compared to the intravenous dose of the current study. The measured (labelling period) and predicted
(intervention period) $^{41}$Ca concentrations in the two studies are shown in Supplemental Figure 3 and the vertical shift is due to the differences in bioavailability of the study doses. Subjects labelled in the fall (full diamonds) tend to show faster elimination rates of $^{41}$Ca after 250 days, that is, between spring and summer, when low serum 25(OH)D levels rapidly increase due to increasing sunlight exposure.
Supplemental Table 1 Model finding for serum 25(OH) vitamin D pharmacokinetics in 24 postmenopausal women who received oral supplementation with cholecalciferol using NONMEM\textsuperscript{1}.

<table>
<thead>
<tr>
<th>Model</th>
<th>ΔOF</th>
<th>Parameter Estimates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ΔOF</td>
<td>CL</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>One CMT, linear, Eta on CL</td>
<td>0</td>
<td>0.98</td>
</tr>
<tr>
<td>One CMT, linear, Eta on CL and V\textsubscript{1}</td>
<td>0</td>
<td>0.98</td>
</tr>
<tr>
<td>… first order absorption for 25(OH)D by sun exposure</td>
<td>0</td>
<td>0.98</td>
</tr>
<tr>
<td>… bioavailability (F) for 25(OH)D by diet and sun exposure estimated</td>
<td>-1</td>
<td>1.00</td>
</tr>
<tr>
<td>Two CMTs, linear, Eta on CL and V\textsubscript{1}</td>
<td>-20</td>
<td>1.04</td>
</tr>
</tbody>
</table>

\textsuperscript{1}One and two compartment models are compared in regard to the change in objective function (ΔOF) and parameter estimates. The linear model with one compartment and inter-individual variability on clearance and V\textsubscript{1} (in bold) was selected as preferred vitamin D model.

* fixed to value. F (supplement) fixed to 1.

Add. error: additional standard error
CL: clearance
CMT: compartment
CV: coefficient of variation
Eta: interindividual variability
F: bioavailability
F(d): F(diet)
F(s): F(synthesis)
k_a: absorption rate (first order)
OF: objective function
Prop. error: proportional standard error
Q: distribution rate
V_1: central volume of distribution
V_2: peripheral compartment volume
Supplemental Table 2 Parameters of the selected calcium model generated by including data from the previously published study (3) with oral labeling, together with data from this study (intravenous labeling) up to administration of vitamin D supplement (mean ± SE)\(^1\).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Estimates</th>
<th>Interindividual Variability</th>
</tr>
</thead>
<tbody>
<tr>
<td>(k_{10}) (d(^{-1}))</td>
<td>0.0617 ± 0.0040</td>
<td>0.0245 ± 0.0054</td>
</tr>
<tr>
<td>(k_{12}) (d(^{-1}))</td>
<td>0.0426 ± 0.0035</td>
<td>0.0536 ± 0.0158</td>
</tr>
<tr>
<td>(k_{21}) (d(^{-1}))</td>
<td>0.0125 ± 0.0015</td>
<td></td>
</tr>
<tr>
<td>(k_{23}) (d(^{-1}))</td>
<td>0.0181 ± 0.0009</td>
<td>0.0230 ± 0.0139</td>
</tr>
<tr>
<td>(k_{32}) (d(^{-1}))</td>
<td>0.00420 ± 0.00046</td>
<td>0.0534 ± 0.0235</td>
</tr>
<tr>
<td>(V_1) (L)</td>
<td>18.9 ± 0.6</td>
<td>0.0155 ± 0.0060</td>
</tr>
<tr>
<td>(F)</td>
<td>0.269 ± 0.018</td>
<td></td>
</tr>
<tr>
<td>prop. error (CV%)</td>
<td>0.102 ± 0.009</td>
<td></td>
</tr>
</tbody>
</table>

\(^1\)The model contains three compartments and due to the missing effect of inter-individual variability on \(k_{21}\), no variability was included on \(k_{21}\).

\[ k_{12} = \theta_i \times e^{\eta_{ij}} \times (1 + E_{max} \times C_D / (C_D + C_{50})) \]

- \(C_{50}\) concentration at half-maximal effect
- \(C_D\) predicted concentration from supplement in vitamin D compartment
- \(CV\) coefficient of variation
- \(E_{max}\) maximal effect
- \(F\) bioavailability
- \(k_{10}\) elimination rate
- \(k_{12}\) distribution rate
- \(OF\) objective function
- prop. proportional
- \(SE\) standard error
- \(V_1\) central volume of distribution
- \(\theta_i\) estimated fixed effect parameter
- \(\eta_{ij}\) estimated random effect parameter by subject (j = 1 to 24)
Supplemental Figure 1

Individual predictions (solid lines) and population predictions (dashed lines) for serum 25(OH)D using the selected one-compartment vitamin D model. Shown are serum 25(OH)D concentrations in three representative postmenopausal women with a poor (O), average (X), and good fit (G), selected at the 10th, 50th, and 90th percentile of the mean squared difference between observed (squares) and predicted (solid line) serum 25(OH)D levels. Vitamin D supplementation is shown schematically in μg/day.
Supplemental Figure 2

Measurements (squares) and individual predictions for plasma $^{41}$Ca in pmol/L for all 24 subjects (each shown separately, A-X). Predictions used fixed parameter estimates and inter-individual variabilities as described by the model leading to Table 2, and individual errors as generated by that model. Fine lines represent individual predictions without taking vitamin D into account, while thick lines show individual predictions using a model in which vitamin D from supplement is allowed to have an effect on $k_{12}$ (Table 5, rows 1 and 3). Dashed lines represent serum 25(OH) vitamin D concentrations in µg/L in the vitamin D compartment as a result of supplementation.
Supplemental Figure 3

$^{41}\text{Ca}$ concentrations in plasma (calculated from $^{41}\text{Ca}/^{40}\text{Ca}$ isotope ratios in urine) after intravenous (triangles, this study) and oral (diamonds) administration of a single dose of $^{41}\text{Ca}$ given to postmenopausal women, and corresponding population predictions for $^{41}\text{Ca}$ administration given orally (solid line) and intravenously (dashed line). Open symbols represent data points from women labeled in the spring, solid symbols represent data points from women labeled in the fall.